

APPLICATION OF COMPUTER TOMOGRAPHY IN ANIMAL BREEDING: A REVIEW

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SUMMARY

Animal body composition is typically estimated by two methods, which either define the chemical composition of the body or the anatomical distribution of its tissue. The technique assessing body composition can either be destructive or non-destructive to the animal. The disadvantages of the former method are the small number of observations possible and the fact examinations can be carried out only once on the same animal. The non-invasive imaging technique like computer tomography (CT) could establish a valuable tool for the determination of body composition performed in series on living animals. The aim of the paper is to overview the different computer tomography applications in animal breeding. In addition to its estimation of animal body composition, CT has currently utilized to predict body composition changes during growth and pregnancy and make genetic evaluation to improve animal production. With the advantages of CT, this technique will in the future most probably develop not only for research but also a wide field application.

ÖSSZEFOGLALÁS

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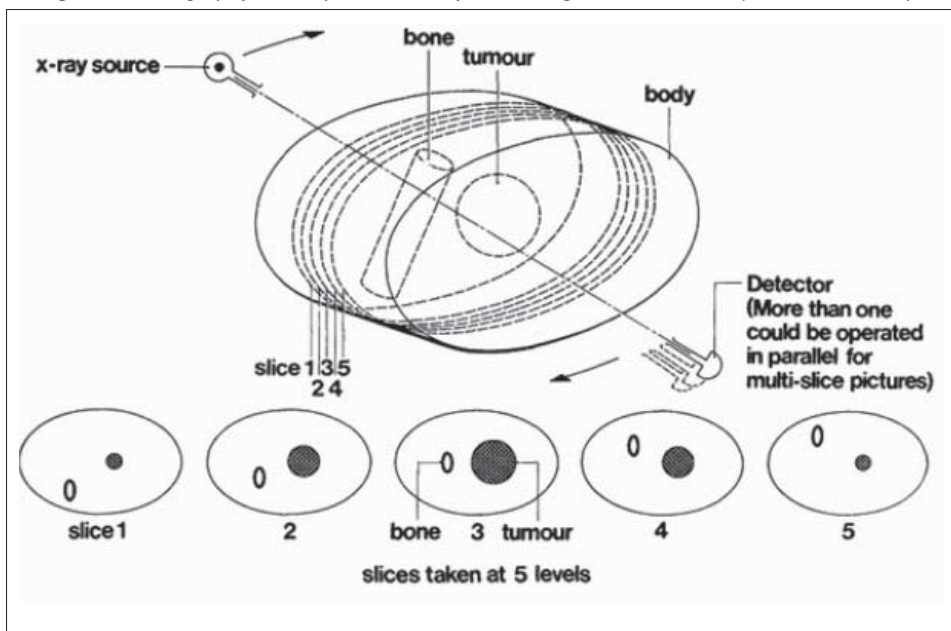
Az állatok testösszetételének becslését általában kétféle módszer alapján végezzük, melyek a kémiai összetételt vagy a szövetek anatómiai eloszlását adják meg. Az alkalmazott technikától függően az állat levágása szükséges lehet, ebben az esetben azonban a vizsgálatot egy adott állaton csak egyszer lehet elvégezni, továbbá a minta nagysága csak korlátozottan növelhető. A *nem invazív* technikák (pl. computer tomográfia - CT) alkalmazása lehetővé teszi, hogy a testösszetételt élő állatokon határozzuk meg. Dolgozatunk célja, hogy áttekintést adjon a CT különböző állattenyésztési alkalmazásairól. A testösszetétel egyszeri becslése mellett a CT eljárást a növekedés, illetve vemhesség alatti testösszetétel változásának nyomonkövetésére használják, továbbá a vizsgált tulajdonságok genetikai értékelésével az állati termelés színvonala is javítható. A CT technika előnyei miatt a jövőben az eljárás kutatási célú alkalmazása mellett a széles körű gyakorlati alkalmazás várható.

INTRODUCTION

Computer tomography (CT) is a technological revolution of in vivo non-invasive diagnostics with the representation of cross-sectional imaging that can be used for detailed measurements of different tissues. Organs, anatomical structures and main tissues can be visualized in the CT images, based on the X-ray attenuation differences among body tissues. The CT scanning was first performed in 1967 and originally used in diagnostic medicine for humans (*Hounsfield, 1973*). Several years later in the early 1980s, CT was realized to be a valuable tool for prediction of the body composition of animals (*Skjervold et al., 1981*). In animal breeding the greatest advantage of this technology is that evaluation of body composition does not require the use of test slaughters. Thus instead of progeny tests - that are expensive and lengthen generation interval - self performance tests can be used. However, it has to be noted that because of the unpredictable characteristics of gene combinations the precision of the progeny test is always higher than that of the self performance test. Using latter method higher selection response may be achieved but the standard deviation of the estimates is also larger. During the recent years in the European Union CT scanning became the official reference procedure determining the lean meat content of pigs on which selection is based in some countries (*Gjerlaug-Enger et al., 2012*). Besides, CT is also widely used for multiple measurements on the same animals monitoring body composition changes during growth and/or pregnancy. Establishing allometrical and growth functions are also closely related to multiple CT measurements. The objective of this study is to present an overview about the different computer tomography applications in animal breeding.

COMPUTER TOMOGRAPHY BASICS

CT uses X-rays to generate cross-sectional, two-dimensional images of the body and each image is acquired by rapid rotation of the X-ray tube 360° around the body of the animal. The object being scanned is divided up into spatially consecutive and parallel sections, the data from which are then summed up to produce total estimate of the different tissues (*Krause, 1999*). The amount of radiation transmitted through the body depends on the attenuation rate of the X-rays, which differ between the various tissue types according to their relative densities (*Bünger et al., 2011*). A large amount of these attenuation values registered by one or multiple arrays of detectors (single slice and multi slice scanners) are managed by one computer. This permits the spatial relationship of the radiation-absorbing structures within it to be digitally established. The image obtained consists of a matrix of attenuation values which are depicted in various shades of grey, thereby creating a spatial image of the scanned object (*Wegener, 1993*). The aim of the system is to produce a series of images by tomography method as illustrated in *Figure 1. (Hounsfield, 1973)*.

Figure 1. Tomography techniques on a body containing bone and tumor (Hounsfield, 1973)**1. ábra** Tomográf technikák alkalmazása csontot és tumort tartalmazó testen

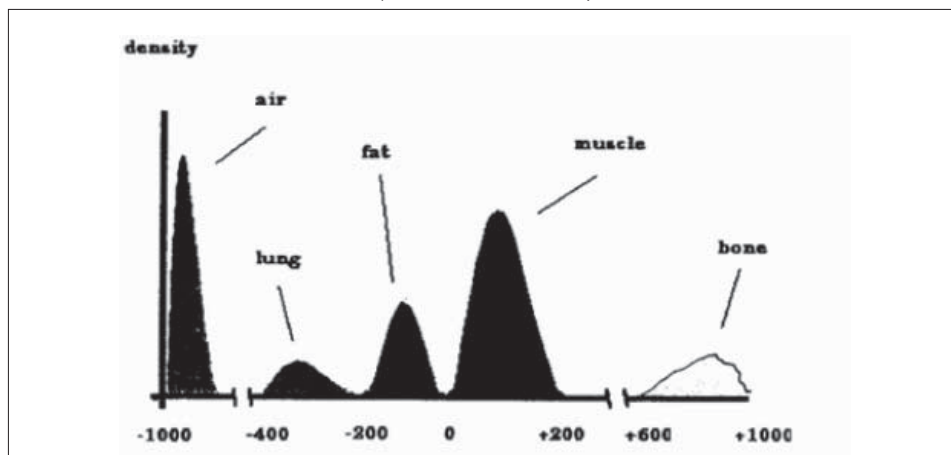
X-ray computed tomography is a non-invasive technique that measures the radio density through the material and is distinguished on the Hounsfield scale. This scale ranges from -1000 (zero absorption) to +1000 (total absorption), and the accepted zero point is the density value of water (0 HU). Fat tissue is usually around -200-0 HU, meat tissue around 0- +200 HU and bone tissue above +600 HU. The CT-volume consists of discrete volume elements (voxels) and is not necessarily isotropic. The entire scale and the absorption range of the various tissue types are illustrated in Figure 2. (Romvári et al., 1996a).

The pixels are the smallest unit of a CT scan. Mostly the CT image is a matrix of 256×256 or 512×512 elements (pixel), depending on the instruments' scan bore and zoom factor used (for example: 0.95 mm^2 at 1.0 zoom factor on Siemens Somatom Plus 40 CT scanner). Principally a cross-section image of a body can be expressed by a certain slice thickness (1-10 mm usually). This shows that actually the pixel is a three dimensional object. The HU values of a pixel calculated by the scanner is the weighted average of the tissue's Hu value found in this volume (Szabó et al., 1999).

The two main approaches used in the evaluation of the CT scan are the so called direct volumetric prediction and the development of prediction equations. With the first method the CT and carcass based percentages are compared by simple correlation and this method has the advantage compared to the predictive equations that it does not have to be re-evaluated regularly with the passing time.

Development of the prediction equation is based on some kind of regression. The simplest possibility is the multiple linear regressions (MGLH) where the variable selection is generally performed by the stepwise procedure. However when the number of variables are far more numerous than the number of observations then

Figure 2. The X-ray absorption values of the various tissues on the Hounsfield scale (Romvári et al., 1996a)



2. ábra Különböző szövetek röntgensugár elnyelő képességének Hounsfield skálán mért értékei

the principal component (PC) or the partial least square (PLS) regressions are preferable. These methods solve the problem of multicollinearity and as noted by Font i Furnols et al. (2009) PLS regression has also the advantage that it is not necessary to classify the voxels into meat or fat avoiding the problem of partial volume effect when in one voxel there are more than one class of tissue.

PREDICTION OF BODY COMPOSITION

When the purpose of the CT scanning is simply to predict body composition then the examined animals are scanned only once. The main tissues to be estimated are muscle and fat tissues. The results of the different studies are summarized in Tables 1-2.

Looking the results in Tables 1-2. it can be seen that the precision of the CT scanning is varying across the different species. However, it has to be noted that the results of the different studies cannot directly be compared not only due to the different characteristics of the examined species (body size, live weight, muscle content etc.) but also because of the reported differences of these studies (status of the scanned animal, the applied reference and the different prediction methods) (Tables 1-2.).

Skjervold et al. (1981) are pioneers to utilize the possibilities of CT for lean content prediction in pig carcasses. In this study the prediction of the protein content of the carcass was performed based only on one scan (tomographic slice taken just behind the last rib) achieving reasonable precision. The percentage of fat could be estimated with higher precision than that of the protein. However, Skjervold et al. (1981) noted that this finding was the consequence of the increased variation in fat content of the animals as they came from lines of pigs selected for high and low backfat thickness. This may caused the overestimation of R^2 values.

Concerning pig breeding in the EU, in most countries tools to predict carcass composition for grading and classification of carcasses are calibrated on the basis of manual dissection references made by butchers. However, this manual technique is limited, time consuming, costly and requires highly skilled butchers (Picouet *et al.*, 2010). The difference in the determination of the lean meat percentage by dissection between butchers was the maximum at 1.96% with an average of 0.98% (Nissen *et al.*, 2006). During the last decade several studies (Romvári *et al.*, 2006; Font i Furnols *et al.*, 2009; Vester-Christensen *et al.*, 2009; Picouet *et al.*, 2010) used CT measurements on pigs with the aim to replace the previous EU Reference method (Council Regulation No. 3220/84) which is based on a standardised jointing of the left carcass side, followed by a tissue dissection of the main parts (Romvári *et al.*, 2006). Compared to the trial of Skjervold *et al.* (1981) (average body weight 57.8 kg) CT analyses were performed on much larger animals (carcass weight ranged between 69.8 and 145 kg) and instead of in vivo measurements half carcasses were scanned and the achieved coefficients of determinations were substantially higher. Comparing the results of these studies (Romvári *et al.*, 2006; Font i Furnols *et al.*, 2009; Vester-Christensen *et al.*, 2009; Picouet *et al.*, 2010) to each other it can be seen that PLS regression was slightly superior to direct volumetric prediction except when the problem of mixed voxels are treated by Bayesian statistics (2D contextual classification) where the neighbouring voxels are also taken into account classifying the tissues into classes (Vester-Christensen *et al.*, 2009) (Table 1.).

Table 1.

Accuracy of CT for estimating muscle tissue percentage				
Species (n)	Reference	Method	R ²	Source
Pig/ <i>in vivo</i> /23	Chemical analysis	LS linear regression	0.83	Skjervold <i>et al.</i> 1981
Pig/ <i>in vivo</i> /130	Dissection	Volumetric estimation	0.94	Romvári <i>et al.</i> 2005
Pig/carcass/60	Dissection (Kulmbach)	Volumetric estimation	0.93	Romvári <i>et al.</i> 2006
		PLS regression	0.99	
Pig/carcass/123	Dissection (simplified)	PLS regression	0.96	Font i Furnols <i>et al.</i> 2009
Pig/carcass/299	Dissection (29)	Volumetric estimation	0.99	Vester-Christensen <i>et al.</i> 2009
Pig/carcass/122	Dissection	Linear regression	0.92	Picouet <i>et al.</i> 2010
Rabbit/ <i>in vivo</i> /406	Chemical analysis	PCA	0.62	Romvári <i>et al.</i> 1998
Rabbit/ <i>in vivo</i> /40	Chemical analysis	MGLH	0.36	Pekete <i>et al.</i> 2005
Rabbit/ <i>in vivo</i> /40	Chemical analysis	MGLH	0.79*	Pekete <i>et al.</i> 2005
Sheep/ <i>in vivo</i> /30	Dissection	MGLH	0.81	Lambe <i>et al.</i> 2003
Sheep/ <i>in vivo</i> /41	Dissection	Linear regression	0.63	Junkuszew and Ringdorfer 2005
Sheep/ <i>in vivo</i> /155	Dissection	Stepwise regression	0.93	Kvame and Vangen 2006
Sheep/ <i>in vivo</i> /240	Dissection	Linear regression	0.97	Navajas <i>et al.</i> 2006
Sheep/carcass/120	Dissection	NPLS regression	0.96	Johansen <i>et al.</i> 2007
Sheep/ <i>in vivo</i> /240	Dissection	MGLH	0.79	Lambe <i>et al.</i> 2003
Sheep/carcass/119	Dissection	Volumetric estimation	0.95	Kongsro <i>et al.</i> 2008
Fish/fillet/48	Chemical analysis	PCA	0.87	Romvári <i>et al.</i> 2002

1. táblázat CT-vel becsült izomszövet arány becslési pontossága

Table 2.

Accuracy of CT for estimating fat tissue percentage					
Species/method/n	Reference	Method	R ²	r	Source
Pig/ <i>in vivo</i> /23	Chemical analysis	LS linear regression	0.89		<i>Skjervold et al.</i> 1981
Pig/ <i>in vivo</i> /130	Dissection	correlation		0.95	<i>Romvári et al.</i> 2005
Rabbit/ <i>in vivo</i> /60	Chemical analysis	PCA	0.90		<i>Romvári et al.</i> 1996c
Rabbit/ <i>in vivo</i> /406	Chemical analysis	PCA	0.92		<i>Romvári et al.</i> 1998
Rabbit/ <i>in vivo</i> /40	Chemical analysis	MGLH	0.87		<i>Fekete et al.</i> 2005
Sheep/ <i>in vivo</i> /30	Dissection	MGLH	0.98		<i>Lambe et al.</i> 2003
Sheep/ <i>in vivo</i> /41	Dissection	Linear regression	0.84		<i>Junkuszew and Ringdörfer</i> 2001
Sheep/ <i>in vivo</i> /155	Dissection	Stepwise regression	0.96		<i>Kvame and Vangen</i> 2006
Sheep/ carcass/120	Dissection	NPLS regression	0.97		<i>Johansen et al.</i> 2007
Sheep/ <i>in vivo</i> /240	Dissection	MGLH	0.80		<i>Lambe et al.</i> 2008
Sheep/ carcass/119	Dissection	Correlation		0.99	<i>Kongsro et al.</i> 2008
Fish/ fillet/48	Chemical analysis	PCA	0.88		<i>Romvári et al.</i> 2002
Fish/ fillet/50	Chemical analysis	Linear regression	0.85		<i>Kolstad et al.</i> 2004

2. táblázat CT-vel becsült zsírszövet arány becslési pontossága

Compared to studies carried out with pigs it can be seen that the precision of muscle and fat prediction was much lower using rabbits (*Romvári et al.*, 1998; *Fekete et al.*, 2005) (Tables 1-2.) and fish (*Romvári et al.*, 2002). However there are several reasons causing this difference from which the most important is probably the much smaller size of these animals. Besides both rabbit studies scanned live animals which lead to lower precision. The number of scans were also much lower especially for *Fekete et al.* (2005) where the achieved precision was low although including body weight in the prediction equation substantially improved precision (Table 1.).

There are good evidences that CT images provide accurate estimations of body composition in sheep (*Navajas et al.*, 2006; *Johansen et al.*, 2007). *Kongsro et al.* (2008) performed the virtual dissection of lamb carcasses by CT and its correlation to manual dissection. According to the results virtual dissection was more precise and reliable than manual. Additionally, coefficient of determination for weight of lean, fat and bone depends on body region (shoulder, mid-region and leg) estimated by CT (*Kvame and Vangen*, 2006). Although lean and fat content of each body region is different, the accuracy of the CT method used in each area is quite high both for lean 0.89-0.93 and for fat 0.95-0.98, respectively.

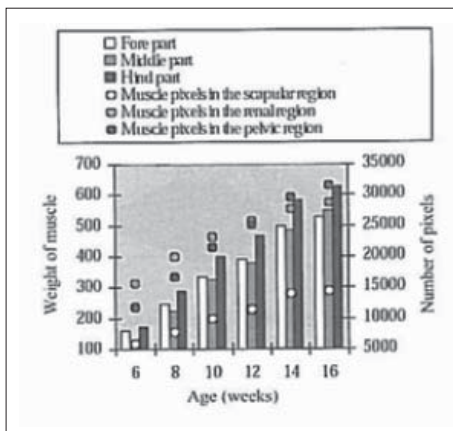
Computer tomography not only provides a high accuracy in estimating body composition of small animals, presents a reliable result in determining the corresponding composition of large animals like cattle. *Holló et al.* (2007) carried out the series of experiments to examine the opportunity for application of X-ray computer tomography in cattle production. Results indicated that lean meat

content of rib samples of the same slaughter cattle analyzed by CT showed a close correlation ($r = 0.97$) with the actual lean meat content of carcasses. Similar results were reported by Navajas *et al.* (2010a; 2010b). In another study Holló *et al.* (2011) also showed the possibilities of the combined use of CT and ultrasound measurements to estimate beef carcass value objectively. For other important traits in beef cattle such as intramuscular fat content successful application of microcomputed tomography was reported by Frisullo *et al.* (2010).

MONITORING BODY COMPOSITION CHANGES

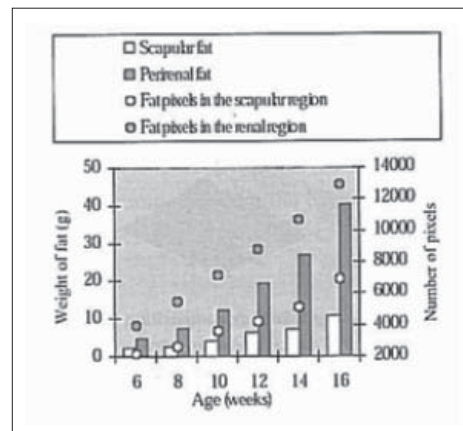
Computer tomography is also used to estimate the tissue development of animals during growth and pregnancy. Studying the growth of rabbits Romvári *et al.* (1996b; 1996c) and Milisits *et al.* (2000) scanned the growing rabbits at 5 consecutive times either at a predefined weight (from 0.5 to 3.5 kg) or age (from 6 to 16 weeks) range. In order to demonstrate the body composition of the rabbits at different developmental stages three-dimensional histograms were created by the method of negative exponential interpolation. To show the changes in the body composition between two examined time points differential histograms were plotted by subtracting the former from the latter (Romvári *et al.*, 1996b; Milisits *et al.* 2000). According to the results, growth of muscle seems to be steady in the first half of the experimental period and decreases only after the 12th week of age (Milisits *et al.* 2000) (Figure 3.). Similarly according to Romvári *et al.* (1996b) between the body weights of 0.5 and 1.5 kg almost exclusively muscle tissue is being built in. On the contrary the very intensive fat deposition begins in the second half of the examined period (Milisits *et al.* 2000) and an abrupt rise in fat content was observed 2.5 kg (Figure 4.). As mentioned by Romvári *et al.* (1996c) between the ages of 6 and 16 weeks the interval of the crude fat doubled, while

Figure 3. Changes in the amount of muscle between 6 and 16 weeks of age (Milisits *et al.*, 2000)



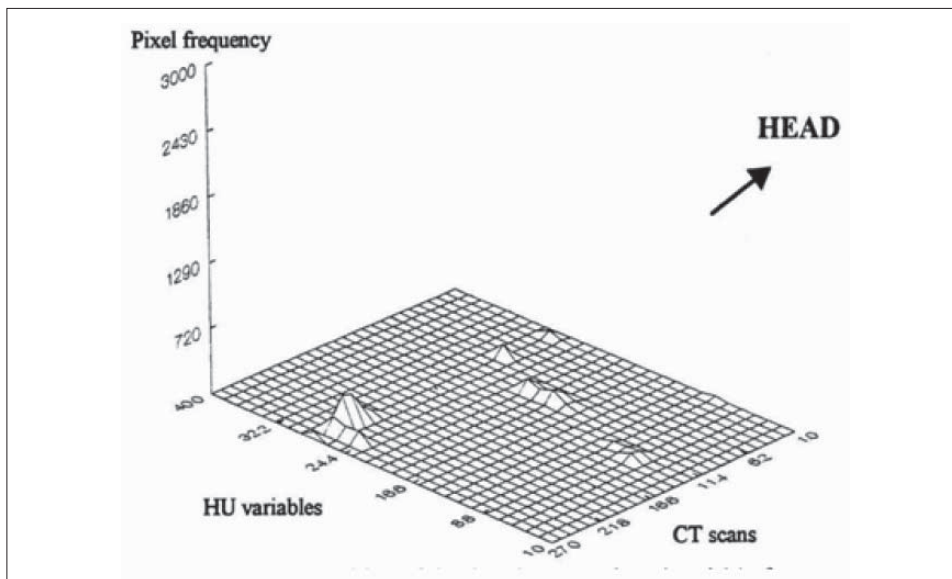
3. ábra Az izomszövet mennyiségének változása 6 és 16 hetes kor között

Figure 4. Changes in the amount of fat between 6 and 16 weeks of age (Milisits *et al.*, 2000)



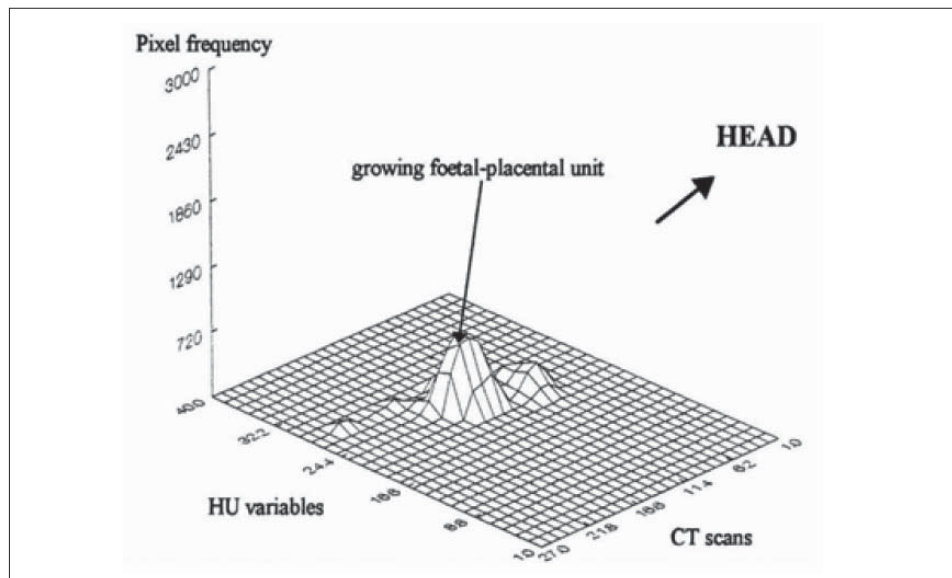
4. ábra A zsírszövet mennyiségének változása 6 és 16 hetes kor között

Figure 5. Changes in body composition of the does between days 1 and 14 of pregnancy, shown as a difference of two histograms (Milisits et al., 1999).



5. ábra Nyulak testösszetételének változása a vemhesség első két hetében két hisztogram különbségeként ábrázolva

Figure 6. Changes in body composition of the does between days 21 and 28 of pregnancy, shown as a difference of two histograms (Milisits et al., 1999)



6. ábra Nyulak testösszetételének változása a vemhesség negyedik hetében két hisztogram különbségeként ábrázolva

the perirenal fat underwent a 5.5 fold increase. The correlations between these traits was high ($R^2 = 0.84$) which suggests that the weight of the perirenal fat is quite a good indicator of total body fat content.

The body composition changes during pregnancy were studied by several authors (*Milisits et al.*, 1996; 1999; *Fekete et al.*, 2005). The main difference between these authors was that *Fekete et al.* (2005) used test slaughters in order to control the precision of the CT measurements *Milisits et al.* (1996; 1999) used only CT measurements which gave the advantage that the body composition changes were monitored on the same animals rather than on different samples (*Fekete et al.*, 2005). *Milisits et al.* (1996; 1999) scanned the female rabbits at 5 different times (at AI, days 14, 21, 28 of gestation and few hours after kindling). In order to show the body compositions and their changes they used the same type of three-dimensional histograms as was shown for growing rabbits (*Milisits et al.*, 2000). According to the results little difference was seen during the first 14 days of pregnancy (*Figure 5*). Whereas, during the last third an intensive mobilization of fat took place in the maternal body to support the accelerated growth of the fetuses (*Figure 6*).

GENETIC EVALUATION OF CT MEASURED TRAITS

Researchers in animal science have for a long time sought an accurate and reliable method of measuring *in vivo* body composition, mainly with the aim to improve and manage carcass composition. Improving carcass composition can be done by manipulating the environment, primarily through quantity and quality of nutrition (*Emmans et al.*, 2000). However, there are strong reasons why significant emphasis should be put on altering the genetic potential of animals for growth and development of their carcass tissues (*Bünger et al.*, 2011). Traditional selection, whereby the best animals are kept as parents for the next generation, offers permanent, cumulative gains that fit well within a sustainable livestock production system. Coupled with modern statistical approaches to breeding scheme design and genetic analysis, substantial genetic gains are possible (*Simm*, 1998).

The first CT-aided selection in animal science is carried out with rabbits using the Pannon White rabbit population of the Kaposvár University, Hungary. The first stage of examination aimed at selection for improved slaughter value of Pannon White rabbits. The determination was made of the sectional planes in which the CT data recorded are suitable for estimation. It was reported that the phenotypic correlations between the average cross section of the m. *Longissimus dorsi*, the so called L-value (between the 2nd and 3rd and 4th and 5th lumbar vertebrae based on *in vivo* computerized tomography) and the dressing out percentage was 0.71 (*Szendrő et al.*, 1992). In a divergent selection experiment *Szendrő et al.* (1996) used a very simple approach regressing the L-value on live body weight and selecting the animals on the residuals. After two generations the difference was 5% and 2% in the L-value and dressing out percentage, respectively between the up and down selected rabbits. After some years of intermission CT selection started again in 2000 and practiced continuously ever since. Since 2003 CT data evaluated with REML and BLUP animal models. The favourable association between the L-value and dressing out percentage was also reported by Nagy

et al. (2006) using Bayesian methods ($r_g = 0.47$) and the heritability of the L-value was moderate (0.33). Contrary to the favourable results after 2004 the selection criterion of L-value was changed to thigh muscle volume (based on in vivo computerized tomography) because its weight is 2.5 times larger than that of the L-value thus as a result increased meat production was expected. Based on the genetic evaluation of *Nagy et al.* (2010) the thigh muscle volume has a moderately low heritability (0.19) but shows a favourable genetic correlation (0.59) with the hind part percentage thus it is expected that the weight of the valuable cuts will be increased. This expectation was justified by *Szendrő et al.* (2012) in a divergent selection experiment where the rabbits were selected for increased and decreased thigh muscle volume. After two generations the rabbits in the first groups showed significantly higher thigh muscle volume (336 vs. 309 cm³), better feed conversion ratio (2.81 vs 3.01 kg) and the ratio of the hind part was also more advantageous in the plus selected group (38.2 vs. 36.3%). However, according to the recent study of *Gyovai et al.* (2012) thigh muscle volume showed an unfavourable genetic correlation with litter weight at day 21 especially at the 3rd (-0.53) and 4th (-0.70) parities which shows that selection of increased muscle production may also have negative effects on reproductive performance.

Heritability estimates for all live sheep measurements were examined by *Karamichou et al.*, (2006 and 2007). Results published by *Karamichou et al.* (2007) showed that heritability for traits describing carcass composition was all moderate, and their maternal genetic component was small or not significant ($p > 0.05$). In contrast, for live weight at scanning, the maternal genetic contribution and the contribution of the genes of the lamb were very similar (0.16 and 0.17, respectively). The CT index was highly heritable (0.41), which is in consistent with selection on the index being successful, indicating that CT provides a quick and reliable means of genetically changing carcass composition in sheep. In another study, on a different population of Blackface sheep at 24 weeks of age (*Karamichou et al.*, 2006), heritability estimates of CT tissue areas traits were moderate to high and were very similar for bone (average $h^2 = 0.36$) and muscle areas (average $h^2 = 0.33$), but somewhat higher than the current study for the three fat areas (average $h^2 = 0.64$). Relationships of the CT index with live weight at scanning and muscle depth were moderately strong, indicating CT measurement provides an effective means of selecting for improved carcass composition and shows high levels of genetic variation, which is the second requirement for making genetic progress. Concerning CT application it has to be noted that CT scanning is much more expensive and also more complicated than the ultrasound measurements therefore if the trait which is measured by CT shows high genetic correlation with another trait measured by ultrasound and the heritability of the latter traits is not substantially smaller than the profitability of CT, CT measurement is doubtful. *Maximini et al.* (2012) reported that the eye muscle area had even lower heritability than that of the eye muscle depth (measured by ultrasound) (0.28) moreover the genetic correlation between these traits were so high (0.84) that these traits practically identical. Fat areas measured by CT showed higher heritabilities (0.36 and 0.40) than fat depth by ultrasound (0.29) but their genetic correlations were also high (0.49 and 0.66). Because of these findings in Australia the CT analysis of the sheep has been stopped in 2011 due to its high costs (and lack of additional

gain). Similar findings were reported by *Kvame and Vangen* (2007) where the estimated genetic correlations between CT estimated lean and fat weight and ultrasound muscle and fat depth were high (0.70 and 0.82).

Computed tomography technology was also used to measure body composition on live pigs for breeding purposes (*Luiting et al.*, 1995; *Kolstad*, 2001, *Gjerlaug-Enger et al.*, 2011). Particularly, Norsvin (the Norwegian Pig Breeders Association) is running a boar-testing station with routine CT scanning of live pigs in a breeding program. According to *Gjerlaug-Enger et al.* (2011) the CT technology used to measure production efficiency yielded moderate-to-high heritability estimates for all analyzed traits in both Duroc and Landrace breeds. In general, Duroc had a higher additive genetic variation for the traits analyzed than Landrace, in addition to higher estimated heritability. The estimated heritability for average daily growth of muscle, average daily growth of carcass fat, average daily growth of bone, average daily growth of non-carcass tissue, and the mass of muscle tissue divided by the total mass of the carcass ranged from 0.19 to 0.53 in Landrace and from 0.43 to 0.59 in Duroc.

CONCLUSION

Computer tomography technology reviewed in this paper may be an alternative to manual dissection depending on the characteristics of the analyzed population. The high values for correlations suggest that prediction of fat and lean would be accurate using this method either measuring body composition at a given time or monitoring body composition changes during growth or pregnancy. Furthermore, CT measurement shows higher genetic variation, which is a requirement for making genetic progress. Thus CT measured traits are also suitable as selection criteria traits and they can be improved by selection.

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